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TITLE: Conversion of non-neuronal cells into neurons: transdifferentiation of epidermal cells

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CLAIMS:

We claim:
1. A method of transdifferentiating an epidermal basal cell into a cell having a morphological, physiological and/or immunological feature of a viable neuronal cell, comprising the steps of:
(a) obtaining an epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin;
(b) transfecting said epidermal basal cell, in vitro, with one or more eukaryotic expression vector(s) containing at least one cDNA encoding a human neurogenic transcription factor, or homologous non-human counterpart, from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, such that at least one of the neurogenic transcription factor(s) is expressed in said cell;
(c) adding at least one antisense oligonucleotide comprising a segment of a human MSX1 gene or human HES1 gene, or homologous non-human counterpart of either of these, to an in vitro growth medium, thereby suppressing at least one negative regulator of neuronal differentiation; and
(d) growing the transfected epidermal cell with a retinoid and at least one neurotrophin from the group consisting of BDNF, NGF, NT-3, and NT-4, or a cytokine comprising IL-6, whereby said epidermal cell is transdifferentiated into a cell that comprises one or more morphological neurite-like processes at least about 50 micrometers in length and expresses at least one neural-specific antigen selected from the group consisting of neurofilament M, neural-specific tubulin, neural-specific enolase, and microtubule associated protein 2.
2. The method of claim 1, wherein the eukaryotic expression vector(s) of the transfection step comprise a CMV promoter sequence operatively linked to a DNA(s) encoding the neurogenic transgenic transcription factor from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, and wherein the DNA encoding the neurogenic transcription factor is of human origin or is a homologous non-human counterpart of a gene encoding any of these.
3. A transdifferentiated cell having a morphological, physiological and/or immunological feature of a viable neuronal cell, comprising:
an epidermal basal cell transfected with one or more expression vectors comprising a CMV promoter sequence operatively linked to a DNA(s) encoding the neurogenic transcription factor NeuroD1, NeuroD2, ASH1, Zic1, Zic3, or MyT1, wherein the DNA encoding the neurogenic transcription factor is of human origin, or is a non-human homologous counterpart or a gene encoding any of these, wherein said cell is treated with at least one antisense oligonucleotide comprising a segment(s) of human MSX1 gene or human HES1

gene, or non-human homologous counterpart thereof, and wherein said cell was grown in the presence of a retinoid and at least one neurotrophin, thereby transdifferentiating said epidermal cell into a cell comprising one or more morphological neurite-like process(es) at least about 50 micrometers in length and expressing at least one neural-specific antigen selected from the group consisting of neurofilament M, neural-specific tubulin, neural-specific enolase, and microtubule associated protein 2.

4. A transdifferentiated cell having a morphological, physiological and/or immunological feature of a viable neuronal cell produced by the process of claim 1.

5. A kit for converting epidermal basal cells to cells that comprise one or more morphological neurite-like processes at least about 50 micrometers in length and express at least one neural-specific antigen selected from the group consisting of neurofilament M, neural-specific tubulin, neural-specific enolase, and microtubule associated protein 2, said kit comprising:

one or more eukaryotic expression vector(s) containing cDNA encoding a neurogenic transcription factor, or fragment thereof, from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, or a non-human homologous counterpart of any of these;

at least one antisense oligonucleotide corresponding to the human MSX1 gene, the human HES1 gene, or a non-human homologous counterpart of either of these;

a retinoid and at least one neurotrophin from the group consisting of BDNF, NGF, NT-3, and NT-4.

6. The kit of claim 5, further comprising instruction for use with a patient's

claims 24-26 are canceled.

7. A method of using transdifferentiated epidermal basal cells having a morphological, physiological and/or immunological feature of viable neuronal cells to isolate a novel nerve growth factor comprising the steps of:

(a) transdifferentiating epidermal basal cells to cells having a morphological, physiological and/or immunological feature of a viable neuronal cell as in claim 1;

(b) culturing the transdifferentiated cells in vitro;

(c) exposing the cultured cells in vitro, to a potential nerve growth factor; and

(d) detecting the presence or absence of an effect of the potential nerve growth factor on the survival of the cells or on a morphological or electrophysiolocical characteristic and/or molecular biological property of said transdifferentiated epidermal basal cells, whereby an effect altering cell survival, a morphological or electrophysiological characteristic and/or a molecular biological property of the cells indicates the activity of the novel nerve growth factor.

8. A method of using transdifferentiated epidermal basal cells having a morphological, physiological and/or immunological feature of viable neuronal cells to screen a potential new drug to treat a nervous system disorder comprising the steps of:

(a) transdifferentiating epidermal basal cells from a patient with a nervous system disorder to cells having a morphological, physiological and/or immunological feature of a viable neuronal cell as in claim 1;

(b) culturing the transdifferentiated cells in vitro;

(c) exposing the cultured cells, in vitro, to a potential new drug; and

(d) detecting the presence or absence of an effect of the potential new drug on the survival of the cells in vitro or on a morphological or electrophysiological characteristic and/or molecular biological property of said transdifferentiated epidermal basal cells, whereby an effect altering cell survival, a morphological or electrophysiological characteristic and/or a molecular biological property of the cells in vitro indicates the activity of the potential new drug.

9. A transdifferentiated epiderinal basal cell having a morphological, physiological and/or immunological feature of a viable neuronal cell comprising:

a cell of epidermal basal cell origin which displays one or more morphological neurite-like process(es) at least about 50 micrometers in length and expresses at least one neural-specific antigen selected from the group consisting of neurofilament M, neural-specific tubulin, neural-specific enolase, and microtubule associated protein 2.

10. The cell of claim 9, wherein the the cell further displays a lack of proliferation in conditions which induce differentiation.

11. The cell of claim 9, wherein the cell is GABAergic.

12. The cell of claim 9, wherein the cell is dopaminergic.

13. A transdifferentiated cell comprising a cell of basal epidermal origin which displays a morphological, physiological and/or immunological feature of an astroglial cell and wherein said immunological feature is expression of glial fibrillary acidic protein (GFAP).

14. The method of claim 1, wherein obtaining an epidermal basal cell comprises selecting basal cells from keratinocytes using a calcium-free medium.
15. The method of claim 1, wherein said antisense oligonucleotide(s) is modified with one or more thio groups.